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Author

Smith, MT

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The Mechanism of Benzene-induced Leukemia: A Hypothesis and Speculations on the Causes of Leukemia

Martyn T. Smith

School of Public Health, University of California, Berkeley, California

An overall hypothesis for benzene-induced leukemia is proposed. Key components of the hypothesis include *a*) activation of benzene in the liver to phenolic metabolites; *b*) transport of these metabolites to the bone marrow and conversion to semiquinone radicals and quinones via peroxidase enzymes; *c*) generation of active oxygen species via redox cycling; *d*) damage to tubulin, histone proteins, topoisomerase II, other DNA associated proteins, and DNA itself; and *e*) consequent damage including DNA strand breakage, mitotic recombination, chromosome translocations, and aneuploidy. If these effects take place in stem or early progenitor cells a leukemic clone with selective advantage to grow may arise, as a result of protooncogene activation, gene fusion, and suppressor gene inactivation. Epigenetic effects of benzene metabolites on the bone marrow stroma, and perhaps the stem cell itself, may then foster development and survival of the leukemic clone. Evidence for this hypothesis is mounting with the recent demonstration that benzene induces gene-duplicating mutations in human bone marrow and chromosome-specific aneuploidy and translocations in peripheral blood cells. If this hypothesis is correct, it also potentially implicates phenolic and quinonoid compounds in the induction of "spontaneous" leukemia in man. — Environ Health Perspect 104(Suppl 6):1219–1225 (1996)

Key words: phenol, hydroquinone, benzenetriol, quinones, free radicals, oxygen radicals, chromosome damage, aneuploidy, recombination, topoisomerase, carcinogenesis, leukemia

Introduction

Benzene is an established human leukemogen, but the mechanism by which it produces leukemia remains unclear. This article presents a hypothesis of how benzene produces leukemia. The hypothesis has a number of themes which postulate that benzene is an unusual carcinogen. For example, I propose that a number of metabolites work in concert to produce its effects and that the genotoxic effects that these metabolites produce are not simple point mutations, but rather recombination and chromosomal aberrations. The article compares benzene to more classical carcinogens and then outlines the hypothesis in detail.

Benzene, Not a "Classic" Carcinogen

Textbooks tend to narrow our thinking as to the way in which chemicals produce cancer. "Classic" carcinogens, such as benzo[*a*]pyrene, aromatic amines, and aflatoxin, are thought to be activated to a single, ultimate carcinogenic metabolite. Typically these metabolites are highly electrophilic and bind strongly to DNA in a covalent fashion. Covalent binding of this type is readily measured as bound radioactivity. Classic carcinogens and their metabolites are also highly mutagenic in the Ames *Salmonella* test producing point mutations and small deletions.

Benzene presents the exact opposite scenario. It does not form a single highly electrophilic metabolite. Its epoxide, benzene oxide, is highly unstable and rapidly rearranges to the major metabolite phenol. Alternatively, it is metabolized via epoxide hydrolase and dihydrodiol dehydrogenase to catechol. None of the prominent metabolites of benzene are "hard" electrophiles and thus little binding to DNA is detected as bound radioactivity when radiolabeled benzene is administered to experimental animals (*1*). Benzene and the majority of its metabolites are also not mutagenic in the Ames *Salmonella* test (*2*). They do, however, produce chromosomal damage both *in vitro* and *in vivo* (*2–5*).

How is Benzene Carcinogenic?

If benzene is not a "classic" carcinogen binding to DNA and causing point mutations, how, then, is it carcinogenic? I propose that benzene is carcinogenic by its phenolic metabolites acting in concert to produce DNA strand breaks, topoisomerase II inhibition, and damage to the mitotic spindle. This leads to mitotic recombination, chromosome translocations, and aneuploidy (the loss and gain of whole chromosomes). These genotoxic events will, in turn, cause the activation of key protooncogenes, loss of heterozygosity, and inactivation of tumor suppressor genes. If this takes place in bone marrow stem or early progenitor cells, a leukemic clone with selective growth advantage could arise. Epigenetic effects of benzene on the bone marrow stroma and progenitor cells could then assist in the establishment of a leukemic clone. Both genetic and epigenetic effects are therefore probably necessary. Figure 1 outlines this hypothesis in more detail.

The Role of Metabolism and the Nature of the Toxic Metabolites

To be carcinogenic, benzene must first be metabolized in the liver, mainly via cytochrome P4502E1. The major product is phenol, which is either conjugated—primarily to phenyl sulfate in humans—or further hydroxylated by P4502E1 to hydroquinone. Other major metabolites include catechol and *trans-trans*-muconic acid. The latter is presumed to be formed from the ring opening of benzene epoxide via benzene oxepin, or perhaps benzene dihydrodiol. The intermediate product *trans-trans*-muconaldehyde has genotoxic properties and could play a role in benzene toxicity (*6,7*). The selective toxicity of benzene to blood and bone marrow would be hard to explain if *trans-trans*-muconaldehyde were

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Address correspondence to Professor M.T. Smith, School of Public Health, University of California, 140 Warren Hall, Berkeley, CA 94720-7360. Telephone: (510) 642-8770. Fax: (510) 642-5815. E-mail: martynts@uclink4.berkeley.edu

Abbreviations used: COMET, single-cell get assay; GPA, glycophorin; MPO, myeloperoxidase.

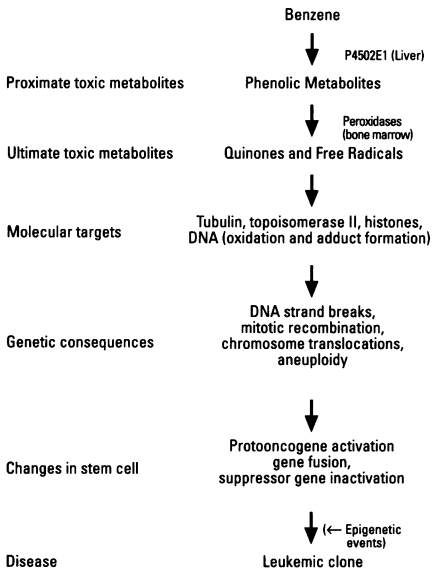

Figure 1. A mechanistic hypothesis of benzene-induced leukemia.

the sole toxic metabolite since it would have to be formed in the liver, travel to the bone marrow, and induce selective toxicity. It is difficult to imagine significant quantities of *trans-trans*-muconaldehyde escaping hepatic glutathione and reaching the bone marrow. Indeed, studies have shown that little or no *trans-trans*-muconaldehyde is likely to leave the liver(*8*).

There is now strong evidence that quinones and related free radicals are the ultimate toxic metabolites of benzene. Specifically, I believe that 1,4-benzoquinone and its semiquinone radical derived from hydroquinone are the most critical toxic intermediates. The conversion of phenol to diphenoquinone and radical intermediates could also play an important role, as could oxidation products of 1,2,4-benzenetriol. The latter, although formed in small quantities, has potent effects, and its formation in higher amounts in mice may partly explain that species' susceptibility to benzene toxicity (*9*). Active oxygen species are produced through autoxidation of 1,2,4-benzenetriol which can cause strand breaks, microtubule damage, micronuclei, and aneuploidy in human cells (*10,11*). The role, if any, of 1,2-benzoquinone derived from catechol remains unclear.

1,4-Benzoquinone and *trans-trans*-muconaldehyde are similar chemically, since they are both α,β-unsaturated diketones. They are therefore likely to hit similar molecular targets and to have similar toxic effects. Indeed, the combination of hydroquinone and *trans-trans*-muconaldehyde has been shown to be highly toxic (*12*).

An argument against the hypothesis that quinones are the ultimate toxic metabolites of benzene has been that their precursors phenol, hydroquinone, and catechol do not produce bone marrow toxicity or leukemia. A logical explanation of this apparent contradiction may be at hand, however, related to the locations of activating and conjugating enzymes in the liver (*13*). The liver is structurally divided into three acinar zones labeled I, II, and III. The primary site of conjugating enzymes is in zone I whereas that of P4502E1 is zone III. Blood flows from the hepatic portal vein and artery first into zone I, then into zone II, and finally into zone III before exiting via a branch of the hepatic vein. Thus, phenols and quinones entering the liver will be conjugated immediately in zone I, while benzene would escape conjugation and go directly to zone III where it would be converted to phenol and hydroquinone and would then leave the liver in both the free and conjugated forms. Far more free, unconjugated phenols would be expected to leave the liver after benzene administration than after exposure to phenol. Indeed, new pharmacokinetic modeling predicts that bone marrow concentrations of free phenol and hydroquinone will be much higher after benzene administration than following phenol exposure. This is the opposite to what we had previously predicted with a pharmacokinetic model that considered the liver a single, homogeneous compartment (*14*).

The new, heterogeneous liver compartment model is likely to be much more accurate, but experimental confirmation will be difficult. This is because any free phenol and hydroquinone reaching the bone marrow is likely to be rapidly oxidized via peroxidase enzymes to protein-binding species. Only when this secondary oxidation pathway approaches saturation at very high benzene levels are there likely to be free phenolic metabolites in the bone marrow. Indeed, recent work by Henderson and colleagues supports this idea (*15*). Predicted metabolite concentrations would therefore have to be measured as bound and free forms.

A Role for Multiple Metabolites

Evidence that multiple metabolites are important in benzene toxicity has increased in recent years. Together with David Eastmond and Richard Irons, I reported that phenol and hydroquinone, when administered together, produced bone marrow toxicity in mice that was very similar to that produced by benzene, whereas alone they did not (*16*). We postulated that peroxidase enzymes in the bone marrow, mainly myeloperoxidase (MPO), were responsible for the secondary activation of benzene's phenolic metabolites to toxic quinones and free radicals (*17*). Further, we showed that phenol would enhance the MPO-dependent oxidation of hydroquinone to 1,4-benzoquinone. We next showed that phenol increased the covalent binding of hydroquinone in bone marrow (*18*) and that catechol would also stimulate the peroxidase-dependent activation of hydroquinone (*19*). Catechol and hydroquinone also produced a synergistic genotoxic effect in human lymphocytes (*20*).

Barale and co-workers have performed experiments in mice that support this idea of an interaction being important, by showing that various combinations of benzene metabolites are genotoxic (*21*). Studies by Bodell and co-workers (*22*) and our group (*23*) also support a multimetabolite mechanism of genotoxicity.

Of great interest is the recent finding that the target organs for benzene carcinogenicity in rodents are rich in both peroxidase and sulfatase enzymes (*24*). The bone marrow, Zymbal gland, and harderian gland are all rich in peroxidases, which can activate phenols to toxic quinones and free radicals. Sulfatases, which remove conjugated sulfate forming free phenols, are also present at high levels in these target organs. The selective distribution of these two types of enzymes in the body may explain the accumulation of free phenol, hydroquinone, and catechol in the bone marrow and the target organ toxicity of benzene.

Molecular Targets of Benzene's Toxic Metabolites

All of the phenolic metabolites of benzene can be oxidized by MPO and other peroxidase enzymes to their respective semiquinone radicals and quinones (*25*). These species are highly toxic by directly binding to cellular macromolecules and generating oxygen radicals through redox cycling. We examined the ability of benzene and its metabolites to produce oxygen radicals and oxidative DNA damage in human cells *in vitro* and in mice *in vivo*. (*23*) We used the human myeloid cell line HL-60, which contains high levels of MPO, for these studies. HL-60 cells were incubated with different benzene metabolites for 30 min,

the DNA isolated and the amount of 8-hydroxy-2′-deoxyguanosine (8-OHdG) measured by high-performance liquid chromatography (HPLC) with electrochemical detection. The formation of 8-OHdG is reflective of hydroxyl radical attack on DNA. We found that phenol, hydroquinone, and 1,2,4-benzenetriol all increased the level of 8-OHdG in HL-60 cells. Catechol did not significantly increase the 8-OHdG level, which is consistent with the limited ability of its quinone oxidation product to redox cycle. We followed up these findings in HL-60 cells with *in vivo* studies in B6C3F1 mice. We administered benzene at various dose levels and for different time periods. We found that benzene (200 mg/kg) produced a highly significant, 5-fold increase in 8-OHdG in bone-marrow DNA 1 hr after administration. This level later decreased to background levels, probably because of repair. A recent study of Italian gas station attendants supports these findings in mice and HL-60 cells by showing that benzene exposure correlated with increased 8-OHdG levels in the urine (*26*). The metabolites most likely responsible for this effect are phenol, hydroquinone, and 1,2,4-benzenetriol. When injected as a single dose into mice, only benzenetriol significantly increased the level of 8-OHdG in bone marrow DNA (*23*). However, various combinations of phenol, catechol, and hydroquinone also significantly increased the 8-OHdG level with phenol plus hydroquinone being the most effective (*23*). This suggests that multiple metabolites play a role in producing benzene-induced oxidative DNA damage in the bone marrow and adds further weight to the hypothesis that multiple metabolites are involved in benzene toxicity.

The formation of 8-OHdG in DNA has been shown to cause point mutations, especially G–T and A–C base substitutions, and may also cause strand breakage if not repaired. Plappert and co-workers (*27*) recently used the single-cell gel (COMET) assay to demonstrate that benzene clearly has the ability to induce strand breakage in mice. Earlier studies that did not find strand breaks by alkaline elution simply looked at inappropriate times (*28*). DNA strand breaks were also increased in a dose-dependent manner in mouse lymphoma cells incubated with benzene and an S9 activating system or with its phenolic metabolites (*29*).

Strand breakage may also result from damage to key proteins associated with the DNA. For example, recent studies using accelerator mass spectrometry have shown that metabolites of benzene bind selectively to the histone fraction of mouse bone marrow cells (K Turtletaub, personal communication). Damage to histone proteins could alter DNA folding and packaging and assist in the production of strand breaks. Another key protein target for the quinone oxidation products of benzene's phenolic metabolites may be topoisomerase II. Chen and Eastmond (*30*) recently presented convincing evidence that 4,4′-diphenoquinone (a phenol oxidation product) and 1,4-benzoquinone (a hydroquinone oxidation product) are potent inhibitors of topoisomerase II, but not topoisomerase I (*31*). This action is similar to the epidophyllotoxins etoposide and teniposide, which are used in cancer chemotherapy. These agents have been shown to be highly mutagenic (*32*) and have been associated with the production of secondary acute myelogenous leukemias (*33*).

Upon binding to topoisomerase II, epidophyllotoxins and perhaps benzene's quinonoid metabolites, convert the enzyme to a DNA poison (*34*). The enzyme functions inappropriately and instead of uncoiling the DNA and removing loops, it can break the DNA strands. Strand breakage resulting from this action has the potential to produce aberrant mitotic recombination through the joining of inappropriate DNA strands. Likely outcomes of aberrant recombination include cell death or the production of stable structural chromosome aberrations, including chromosome translocations, which may alter the differentiation and growth activity of the cell. It was recently shown that chromosome band 11q23 translocations are DNA topoisomerase II cleavage sites (*35*). Translocations involving band 11q23 occur with high frequency in several types of human leukemia, especially cases occurring in infants and young children (*35,36*).

Induction of Mitotic Recombination and Chromosome Translocations by Benzene and Its Metabolites

Evidence that benzene and its metabolites produce mitotic recombination is increasing. *In vitro* studies with mouse lymphoma L5178Y cells showed that benzene was mutagenic at the TK± locus in the presence of rat liver S9, a metabolic activation system (*29*). The metabolites hydroquinone, catechol, 1,2,4-benzenetriol, 1,4-benzoquinone and *trans-trans*-mucondialdehyde were all mutagenic in this test system. The mutagenicity occurred at doses that were also shown to cause DNA strand breaks.

Recently, in a collaborative project with the National Cancer Institute and Chinese and California Environmental Protection Agency investigators, we used the glycophorin A (GPA) gene mutation assay to examine the type of mutations produced by benzene in human bone marrow (*37*). The GPA assay is able to detect a wide spectrum of mutational mechanisms, including point mutations, deletions, and chromosome-wide events or autosomal chromosome interactions, such as mitotic recombination. The GPA assay measures somatic cell mutation frequency in peripheral erythrocytes. Because mature erythrocytes lack a nucleus, mutations expressed in erythrocytes must have occurred exclusively in precursor erythroid cells or stem cells in the bone marrow. Since bone marrow is the target of benzene toxicity, the GPA assay is highly appropriate for mutational studies. The GPA locus codes for a surface protein that exists in two allelic forms, *M* and *N*, on erythrocytes. The assay measures the frequency of variant cells that have lost expression of the *M* form in blood of heterozygous (*MN*) individuals. The variant cells are either the phenotype *NØ* or *NN*. *NØ* cells are thought to arise from point mutation, deletions, or gene inactivation, whereas *NN* cells presumably arise from mitotic recombination, chromosome loss with reduplication, or gene conversion.

We measured GPA mutation frequency in 24 workers heavily exposed to benzene and 23 matched controls in Shanghai, China. We found that benzene caused a highly significant increase in *NN* variant frequency, but not in *NØ*. These results suggest that benzene produces gene-duplicating mutations, but not gene-inactivating ones, at the GPA locus in human bone marrow. The most likely mechanism by which benzene does this is via mitotic recombination. Further studies are needed to confirm this finding and to examine the ability of benzene metabolites to induce mitotic recombination in human cells.

As outlined earlier, the most likely consequence of aberrant recombination caused by benzene metabolites is the production of stable chromosome translocations. These are a common feature of leukemic cells (*38*). The Philadelphia chromosome, which results from a reciprocal translocation between chromosomes 9 and 22, is centrally involved in chronic myelogenous

leukemia (*39*). The most common translocation found in acute myelogenous leukemia is a reciprocal translocation between chromosomes 8 and 21 (t8;21) (*40*). This results in the fusion of the *AML*-1 and *ETO* genes and produces a highly active transcription factor that promotes excessive growth and differentiation paralysis in myeloid progenitor cells (*40–42*). The t(8;21) translocation and translocations at 11q23 are commonly found in myeloid leukemias resulting from treatment with epidophyllotoxins (*43*). Since benzene metabolites appear to act in a similar fashion to epidophyllotoxins inhibiting topoisomerase II, causing DNA strand breaks and structural aberrations, we hypothesized that benzene may also cause the t(8;21) translocation.

We are currently testing this hypothesis using chromosome painting to detect translocations between chromosomes 8 and 21 in the peripheral blood cells of workers exposed to high levels of benzene (*44*). Preliminary results suggest that benzene increases the rate of translocations between 8 and 21 in highly exposed workers (*45*). The t(8;21) has also been observed in conventional cytogenetic analyses of leukemias associated with benzene or other chemical exposures (*46,47*). The production of t(8;21) translocations is therefore likely to be involved in at least one pathway of benzene-induced leukemogenesis.

The Role of Aneuploidy

Another common genetic abnormality found in leukemias is aneuploidy, the loss and gain of whole chromosomes. Numerical changes in C-group chromosomes 6 to 12 and X have been detected in the blood and bone marrow of patients with benzene-induced myelogenous leukemia, myelodysplastic syndrome, and pancytopenia. One of these patients showed a clonal expansion of cells with trisomy 9, and a nonclone was found in another. We have reported that the benzene metabolite, 1,2,4-benzenetriol induces aneuploidy of chromosome 9 in HL-60 cells (*48*). Eastmond and co-workers reported similar findings following exposure of human lymphocytes to hydroquinone (*49*). Elsewhere in this issue (*50*), we report that benzene induces aneuploidy of chromosome 9 in the lymphocytes of exposed workers in a dose-dependent manner. Benzene and its metabolites are therefore able to produce chromosome-specific aneuploidy, which most likely plays a role in leukemogenesis. Also in this volume, Irons and Stillman (*51,52*) argue that clonal cytogenetic abnormalities involving the loss of all or part of chromosomes 5 and 7 are commonly detected in patients who develop myelogenous leukemia after antineoplastic therapy and benzene/solvent exposure. The q arms of chromosomes 5 and 7 are thought to contain key genes, perhaps suppressor genes, involved in myeloid leukemia, and loss of this genetic material could be a critical event. We have therefore developed a procedure that uses fluorescence *in situ* hybridization to detect the loss of 5, 5q31, 7 or 7q in metaphase spreads. We plan to apply this technique on spreads prepared from the population of benzene-exposed workers we began to study in 1992 (*44*). Preliminary results suggest a selective effect of benzene on chromosome 7 (*45*), which would be intriguing if borne out by further analysis because of the proposed role of genes on 7q in leukemogenesis.

There are numerous mechanisms by which chemicals can induce aneuploidy in cells. These include damage to microtubules in the mitotic spindle, kinetochore detachment, and centriole damage (*52,53*). More than 15 years ago, Irons and co-workers showed that 1,4-benzoquinone would selectively target tubulin and disrupt microtubules *in vitro*. Further, they showed that 1,4-benzoquinone was one of the most potent disrupters known and proposed tubulin as a key target in benzene toxicity (*54,55*). Recently, we demonstrated that hydroquinone and 1,2,4-benzenetriol would disrupt the microtubular structure of intact human cells using fluorescent immunocytochemistry with an antitubulin antibody (*48*). A generic mechanism for aneuploidy production by benzene may therefore be microtubule disruption.

It is difficult to imagine, however, how disruption of microtubules would lead to selective aneuploidy. Indeed, it may not. Benzene's quinonoid metabolites may produce random aneuploidogenic on specific chromosomes, but some alterations may be lethal or cause premature apoptosis. Only cells with nonlethal chromosome abnormalities would then survive to be detectable. It seems almost certain, however, that chromosome-specific aneuploidy plays a key role in the development and progression of leukemia, as it does for many other cancers (*52,55*).

Role of Epigenetic Events

It is clear that benzene is a genotoxic carcinogen. However, other epigenetic phenomena may play a role in benzene-induced hematotoxicity. For example, Kalf and co-workers have reported that benzene toxicity to the bone marrow can be prevented by IL-1 administration (*57*). They postulate that hydroquinone is oxidized to benzoquinone, which then inhibits the conversion of pre-IL-1 to the active form, IL-1 (*57*). Further, they propose that this effect occurs within the stromal cells of the bone marrow, preventing the normal maturation of progenitor cells, and that this plays an important role in benzene-induced aplastic anemia. Irons and co-workers (*58*) have also shown an interesting effect of hydroquinone pretreatment on the granulocyte–macrophage lineage of hematopoietic progenitor cells. His group has shown that hydroquinone pretreatment increases the number of colonies formed by recombinant GM–CSF-induced CFU–GM. At concentrations as low as 10^{-9} molar, hydroquinone produces this stimulatory effect. This would suggest that hydroquinone causes the recruitment of hematopoietic progenitor cells into the granulocyte–macrophage pathway. In theory, this recruitment could provide more targets for the genotoxic effects of hydroquinone and other metabolites (*59*). It also has been shown that this phenomenon is specific to chemicals that cause myeloid leukemias. Leukemia may therefore result from benzene inducing both altered differentiation and genotoxic damage leading to a selective growth advantage for immature cells.

Hypothesis Overview

I propose that benzene is an unusual carcinogen that does not produce cancer through simple gene mutations. It may actually represent an example of a separate class of carcinogens that act by a similar mechanism. This class may include diethylstilbestrol, estrogen, and arsenic. I propose that benzene is metabolized in the liver to a variety of metabolites, which travel to the bone marrow and act in concert to produce its toxicity. I believe the phenolic metabolites are the proximate toxic metabolites and that the selective toxicity of benzene is caused by the fact that its target organs are rich in peroxidase and sulfatase enzymes. The phenolic metabolites phenol, hydroquinone, catechol and benzenetriol are all metabolized by peroxidases, such as MPO, to highly toxic semiquinone radicals and quinones. These, I believe are the ultimate toxic metabolites of benzene, which produce the characteristic pattern of benzene toxicity in humans (leukopenia, aplastic anemia, leukemia) and multiorgan carcinogenicity

in peroxidase/sulfatase-rich tissues of rodents (e.g., the Zymbal and Harderian glands). Active oxygen species generated by the redox cycling of quinone metabolites may also play an important role. Of interest is the recent finding that glutathione conjugates of hydroquinone also redox-cycle and these may be involved in benzene toxicity (*59*).

The key molecular targets of quinones and oxygen radicals generated from benzene are most likely tubulin, histone proteins, topoisomerase II, and other DNA-associated proteins. Damage to these proteins would potentially cause DNA strand breakage, mitotic recombination, chromosome translocations, and malsegregation of chromosomes at anaphase to produce aneuploidy. If these effects took place in stem or early progenitor cells a leukemic clone with selective advantage to grow may arise, as a result of protooncogene activation, gene fusion, and suppressor-gene inactivation. Epigenetic effects of benzene metabolites on the bone marrow stroma, and perhaps the stem cell itself, may then foster development and survival of this leukemic clone. Irons and Stillman (*51,58*) provide further insights into the details of these epigenetic events and propose that recombinational/aneuploidy changes in chromosomes 5 and 7, which produce losses of key genetic material, are important in benzene-induced leukemia. Our work supports the hypothesis that changes in chromosome 7 play a key role. It is also possible that the t(8;21) translocation and trisomy are also involved in the development of leukemia from benzene.

We are currently examining the role of changes in chromosome 5, especially in the 5q31 region. There may be a number of genetic pathways to benzene-induced leukemia, but evidence is mounting that the metabolites of benzene produce genetic changes that are known causative factors in myeloid leukemia, i.e., t(8;21) and aneuploidy of chromosomes 7, 8, and 9. We are therefore close to understanding the mechanisms of benzene-induced leukemia, and perhaps will soon have biological markers that are of use in monitoring its effects in exposed humans and predicting future disease outcome.

Implications and Speculations on the Causes of Leukemia

As part of the above hypothesis, I propose that the proximate carcinogenic metabolites of benzene are its phenolic metabolites, phenol, hydroquinone, catechol, and 1,2,4-benzenetriol. These compounds are quite common dietary constituents and many are used in pharmaceutical preparations. For example, hydroquinone and catechol are present in coffee and cigarette smoke at quite high levels; phenol is present in many foodstuffs and is a common ingredient in numerous skin lotions, lip balms, and mild anesthetics; hydroquinone is also present in photographic developer. It is perhaps not surprising that significant background levels of adducts deriving from these compounds are found on human blood proteins and in rodent bone marrow (*61*). These compounds may therefore play an important role in the development of "spontaneous" leukemia in the human population. It is also possible that environmental benzene exposure plays some role in this regard, since its effects may be additional and linear on the background. This is discussed in greater detail elsewhere (*62*).

One must also consider that there are numerous compounds in our diet, herbs, pharmaceutical preparations, etc., that are similar to the phenolic metabolites of benzene. Phenols and quinones are widespread in nature (*63*). Numerous compounds have been found to be inhibitors of topoisomerase enzymes (*32,34*). These compounds could be especially important in producing infant and early childhood leukemias, since these commonly show cytogenetic abnormalities at band 11q23 and t(8:21). As pointed out by Greaves (*36*), *in utero* exposure is the most likely cause of these leukemias. Topoisomerase inhibitors may also contribute significantly to adult leukemia incidence.

In narrowing our search for the causes of human leukemia, which apart from ionizing radiation remain largely unknown, perhaps we should concentrate on phenolics and compounds that affect the function of tubulin/microtubules and topoisomerase II. In this manner we may be able to prevent the more than 80,000 leukemia/lymphoma deaths that occur in the United States each year and the multitude of others that occur elsewhere in the world.

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